REMARKS

After entry of this amendment, claims 1-15 are pending. Applicants respectfully request entry of the above claim amendments as they are believed to put the claims in condition for allowance or, alternatively, in better form for consideration on appeal. Thus, entry under 37 CFR 1.116 is correct. Claim 1 has been amended without prejudice or disclaimer and find support *inter alia* in the original claim. No new matter has been added. A Notice of Appeal has been filed on April 27, 2009 to allow time to consider this response.

Claims Rejections – 35 USC § 103

Claims 1, 4-7, 12-13 and 15 remain rejected under 35 USC § 103(a) as being obvious over Henikoff in view of Zaccolo. Claims 2, 8-11 and 14 remain rejected under 35 USC § 103(a) as being obvious over Henikoff in view of Zaccolo, further in view of Krokan and Short. Claim 3 remains rejected under 35 USC § 103(a) as being obvious over Henikoff in view of Zaccolo, further in Krokan and Short, and further in view of Lutz and Cosstick. Applicants respectfully disagree. However, to expedite prosecution, claim 1 has been amended without prejudice or disclaimer to clarify that the template used in step (iii) is the (-) strand of the master sequence and not the single stranded deletions of (+) strand generated in step (i). Applicants respectfully request reconsideration and withdrawal of the rejections in light of the present amendment and for the following reasons.

To support a *prima facie* conclusion of obviousness, the prior art must disclose or suggest all the limitations of the claimed invention. See *In re Lowry*, 32 F.3d 1579, 1582, 32 USPQ2d 1031, 1034 (Fed. Cir. 1994), see also *Ex parte Alexander*, 86 USPQ2d 1120, 1122 (BPAI 2007) (where the Board reversed the obviousness rejection in part because the Examiner had not identified all the elements of the claim). Here, the claimed limitations (i.e. process steps) are not taught or suggested by the cited references, alone or in combination. As explained in the Response previously filed and in more detail below, none of the cited references, alone or in combination, shows creation of single-stranded fragments with different length as recited in step (i) of the claimed process. Moreover, none of the cited references, alone or in combination, teaches or suggests elongating the single-stranded fragments of different length having at least one universal or degenerate nucleotide incorporated to the full length of the master sequence as recited in step (iii) of the claimed process. Additionally, none of the cited references, alone or in

combination, shows creation of an ordered series of mutants having the same length as the master sequence, the product of step (iv) of the claimed process.

Henikoff discloses a method for generating nested deletions from any fixed point in a cloned insert. The disclosed method is outlined in Figure 1 at page 2962. As illustrated in Figure 1, a single-stranded DNA is obtained with the aid of a helper phage from helper-infected cells and a fully double-stranded molecule is generated by polymerase extension of a primer, leaving a nick or very small gap adjacent to the 5' end of the primer (steps (1)-(2)). Degradation of the synthesized strand by ExoIII at the 3' end of the gap (step (3)) followed by removing of the single strand opposite to the gap with a single-strand specific endonuclease (ss nuclease, step (4)) leads to the generation of double-stranded fragments of various length as nested deletions of the original DNA molecule. These double-stranded fragments are then repaired and ligated into circular plasmids for E. coli transformation (steps (5)-(6)).

The Examiner asserts that practicing steps (1) to (3) as outlines in Figure 1 of Henikoff would achieve step (i) of the claimed process. The Examiner further asserts that Henikoff teaches production of single stranded (+)-strand fragments by using appropriate helper phage, citing to the passage at page 2966. Applicants respectfully disagree.

Step (i) of the claimed process requires the creation of a collection of single-stranded fragments of the (+)-strand of the master sequence, wherein each member of the collection have the same 5'-terminus and have a deletion in the 3'-terminus. Thus, the end product of step (i) is a collection of single-stranded fragments that vary in length as nested deletions of the (+)-strand of the master sequence.

The end product of steps (1)-(3) of Henikoff's method, on the other hand, would produce only a collection of double-stranded fragments that vary in length as nested deletions of the original DNA as a result of the ExoIII deletion and ss nuclease digestion as discussed above. The use of appropriate helper phage, as noted by the Examiner, would generate a single-stranded template, either the (+)-strand or the (-)-strand of the original DNA, as starting material (of step (1)) which would then allow generation of deletion series from either end of the insert. Thus, even if the (+)-strand fragments may exist in a status of "single-stranded fragments" as alleged by the Examiner, those single-stranded (+)-strand fragments would have the same length as the original DNA and not a collection of single-stranded fragments that vary in length as nested

deletions of the original DNA. Accordingly, steps (1) to (3) as outlines in Figure 1 of Henikoff would not achieve step (i) of the claimed process as alleged by the Examiner.

The Examiner further asserts that step (iv) of the claimed process can be achieved by using appropriate helper phage to produce the single-stranded sequence of both the (+) and (-) strands and synthesizing a (-)-strand by using the (+)-strand as a template as taught by Henikoff at page 2966. Applicants respectfully disagree.

By using the (-)-strand of the master sequence as a template to elongate the (+)-strand produced in step (ii) to the full length of the master sequence, the end product of step (iii) of the claimed process would be a collection of single-stranded (+)-strand fragments having the same length of the master sequence. Using the single-stranded (+)-strand fragment so produced as a template to synthesize a complementary (-)-strand in step (iv), the end product of step (iv) of the claimed process is a collection of double-stranded fragments having the same length of the master sequence.

Conversely, as discuss above, the teaching in Henikoff at page 2966 concerns the use of appropriate helper phage to produce either the (+)-strand or the (-)-strand of the original DNA as starting material for polymerase extension and the generation of ExoIII deletion series from either end of the insert (i.e. starting material for step (1)), as it states:

> Using this system, both strands of a single clone can be used for the generation of deletions. Infection with an M13 or f1 helper such as M13K07 to obtain one strand for polymerase extension and with a *Mike* helper to obtain the other strand should allow one to generate deletion series from either end of the insert.
>
> Henikoff at page 2966, left Col., 2nd paragraph (emphasis added).

Thus, even if the (+)-strand of the original DNA is obtained by using an appropriate helper phage and used as a template to synthesize a (-)-strand as alleged by the Examiner, the end product of such synthesis would leave a nick or very small gap adjacent to the 5' end of the primer due to the nature of the polymerase extension (steps (1)-(2)). Because of the existence of the nick or gap, the end product of such polymerase extension would not be a double-stranded fragment having the same length of the original DNA as produced by step (iv) of the claimed process. Accordingly, the use of appropriate helper phage in the method taught by Henikoff would not achieve step (iv) of the claimed process as alleged by the Examiner.

The Examiner additionally alleges that it would not be possible for the claimed process to generate the full length of starting master sequence given the limitation recited in step (i) of claim 1. Office Action at pages 3-4. Applicants strongly disagree with the Examiner's characterization of the claimed process and respectfully submit that the Examiner's above allegation fails to take into account of the limitation as recited in step (iii) of claim 1.

As amended, step (iii) of claim 1 clearly requires that the (+)-strands produced in step (ii) to be elongated to the full length of the master sequence by using the (-)-strand of the master sequence as a template strand for the elongation. Thus, the end product of step (iv) of the claimed process would be exactly the same length of the starting master sequence. This limitation further distinguishes the claimed process from the method taught in Henikoff.

The Examiner acknowledges that Henikoff does not teach step (ii) of claim 1, but relies on Zoccolo for such teaching. Applicants respectfully submit that Zoccolo does not teach or suggest step (ii) of the claimed process.

As discussed in the previous Response, Zoccolo teaches an approach to random mutagenesis of DNA based on PCR amplification in the presence of a mixture of triphosphates of nucleoside analogues. The PCR mutagenesis is to be performed by amplifying a target DNA fragment in the presence of four normal dNTPs (*i.e.* dATP, dGTP, dCTP, and dTTP) and the nucleoside analogues, dPTP and/or 8-oxodGTP. Based on the method taught in Zoccolo, more than one nucleoside analogue is incorporated into the newly amplified DNA strand in a random fashion, which results in more than one nucleotide substitution scattered over the whole polynucleotide sequence. This is further evidenced by Figure 5 at pages 595-596 of Zoccolo, in which each amplified sequence contains more than one nucleotide substitution randomly scattered over the whole molecule.

Conversely, the claimed process is aimed to introduce nucleotide substitution in only one location of any given polynucleotide molecule. This is achieved by step (ii) of the process as recited in claim 1, where the universal or degenerate nucleotide is introduced at the 3'-terminus of the (+)-strands produced in step (i) and not randomly incorporated by PCR amplification of a DNA template. The use of PCR amplification to introduce the nucleotide substitution in Zoccolo's method further distinguishes the claimed process because no PCR amplification is used. Accordingly, it is respectfully submitted that Zoccolo does not teach or suggest step (ii) of

the claimed process as recited in claim 1.

Furthermore, it is well established that under 35 U.S.C. § 103 the Examiner must consider the reference as a whole. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984). In addition, the Examiner cannot selectively pick and choose from the disclosed parameters without proper motivation as to a particular selection. The mere fact that a reference may be modified to reflect features of the claimed invention does not make the modification, and hence the claimed invention, obvious unless the prior art suggested the desirability of such modification. *In re Mills*, 916 F.2d 680, 682, 16 USPQ2d 1430 (Fed. Cir. 1990); *In re Fritch*, 23 USPQ2d 1780 (Fed. Cir. 1992). "[A] patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art . . . it can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements ***in the way the claimed new invention does***." See *KSR International Co. v. Teleflex Inc.*, 1741 82 USPQ2d 1385, 1396 (2007) (emphasis added). Thus, it is impermissible to simply engage in a hindsight reconstruction of the claimed invention where the reference itself provides no teaching as to why the applicant's combination would have been obvious. *In re Gorman*, 933 F.2d 982, 987, 18 USPQ2d 1885, 1888 (Fed. Cir. 1991).

As discussed above, when considering Henikoff as a whole, Henikoff teaches a method to produce nested deletions in polynucleotides. The "mutagenesis" produced by Henikoff's method therefore is deletion, not point mutations. On the other hand, Zoccolo teaches random mutagenesis of DNA by PCR amplification in the presence of nucleoside analogues. The "mutagenesis" produced by Zoccolo's method therefore is point mutations, not deletion. Because Henikoff and Zoccolo teach different concept of mutagenesis with different series of steps, there is no motivation to combine Henikoff and Zoccolo but for an impermissible hindsight reconstruction of the claimed invention.

Because motivation to combine the references is lacking, and because all the limitations of the claims are not taught, Henikoff and Zoccolo, alone or in combination, do not render obvious the subject matter of the claimed process. The remaining secondary references cited by the Examiner do not remedy the deficiency of Henikoff and Zoccolo, alone or in combination, because they were cited merely to provide additional limitations recited in the dependent claims.

For at least the above reasons, reconsideration and withdrawal of the rejections is respectfully requested.

CONCLUSION

For at least the above reasons, Applicants respectfully request withdrawal of the rejections and allowance of the claims. If any outstanding issues remain, the Examiner is invited to telephone the undersigned at the number given below.

A Notice of Appeal and a Petition for Extension of Time under 37 CFR 1.136(a) have been filed on April 27, 2009 with the required fees.

This response is filed within the two-month period for filing an Appeal Brief or a response to the Office Action mailed November 26, 2008 after the filing of the Notice of Appeal dated April 27, 2009. No further fees are believed due. However, if a fee is due, please charge our Deposit Account No. 03-2775, under Order No. 12810-00231-US from which the undersigned is authorized to draw.

Respectfully submitted,

By /s/ Hui-Ju Wu
Hui-Ju Wu, Ph.D.
Registration No.: 57,209
CONNOLLY BOVE LODGE & HUTZ LLP
1007 North Orange Street
P. O. Box 2207
Wilmington, Delaware 19899-2207
(302) 658-9141
(302) 658-5614 (Fax)
Attorney for Applicants

#650587